ALTERNATIVE OSMOLARITY PROTOCOL

MATERIALS:

- 2x LB Media
- PBS
- Sucrose
- Bacteria on Agar plate
- Antibiotic of choice
- ddH_2O

Recipe for 2x LB Media (500 mL)

- 10 g Tryptone
- 5 g Yeast Extract
- 10 g NaCl
- Add ddH_2O up to 500 mL.

Recipe for 10% sucrose (100 mL)

- 10 g D-Sucrose
- Add 100 mL ddH_2O to it.

Recipe for 20% sucrose (100 mL)

- 20 g D-Sucrose
- Add 100 mL ddH_2O to it.

Recipe for 30% sucrose (50 mL)

- 30 g D-Sucrose
- Add 100 mL ddH_2O to it.

Kanamycin 340 µg/mL

- Use 6.8 mL of stock Kanamycin 50 µg/mL.

Recipe for 0%, 5%, 10% and 15% osmo media (200 mL)

- Add 100 mL of sucrose solution to 100 mL of 2x LB media to prepare respective osmo media (Add 100 mL of ddH_2O in case of 0% osmo media).

PROCEDURE:

- Prepare fresh sucrose solution with 0%, 5%, 10% and 15% sucrose.
- Choose sample with similar fluorescent protein as a positive control and sample without fluorescent protein as a negative control.
- Use only 0% and 15% minimal media for both the positive and the negative controls.
- Prepare overnight culture of the bacteria in 1.2 mL osmo media in Eppendorf tubes. Inoculate one fresh colony in each tube and incubate overnight at 37 ^{o}C.
- Do not forget to include negative and positive controls. Check the OD next morning and note it down.
- Dilute the overnight culture using 1:100 dilutions (200 µL of overnight cultured bacteria into 20 mL of respective media in Erlenmeyer flask) and let them grow in the incubator at 37 ^{o}C until they reach OD ~ 0.3. The dilution ratio could be altered to 1:500 or 1:1000.
- Add Kanamycin 340 µg to 1 mL culture to inhibit protein synthesis and immediately put the sample in refrigerator. When all the samples are done, centrifuge all the samples and remove the media. Suspend the cell pellets in 1 mL of PBS and perform fluorescence analysis directly using plate reader. Replace Kanamycin with other antibiotics if your cells contain Kanamycin resistance gene.
- Use excitation and emission length accordingly (depending on the fluorescent protein). An example that we used is the excitation length 580 nm ± 10 and emission length 627 nm ± 30 to measure RFP. The same will go with the controls.
- Do not forget to include negative and positive control during measurement. Use only media as a blank.
- Troubleshoot - Use the same gain for all the samples; do not adjust gain in the settings.
- Repeat the experiment at least three times to have statistical relevance.

DATA ANALYSIS:

Measure the fluorescence and compare with the controls and blank. Plot it as a graph to observe relative change with different osmole concentrations.